Summary in English

The impact of obesity and metabolic complications on the patient and graft outcomes after liver transplantation

Introduction

Obesity, defined as an excessive body accumulation of adipose tissue, which is adversely–related with welfare and medical condition of individuals affected. Obesity constitutes one of the most contemporaneous global health issues with constant upward tendency. Obesity constitutes a complex medical condition which predisposes to the development of multiple comorbidities – among others: DM2, CVD, chronic kidney disease, dyslipidemia, MAFLD; increases the risk of certain malignancies, which taken together produce negative outcomes in terms of morbidity and mortality. Obesity also constitutes one of the key diagnostic criteria of MS. Up until now multiple risk factors have been identified for the obesity development in the general population, among which unhealthy lifestyle appears to predominate recently (positive energy balance, especially in combination with low physical activity, unhealthy eating patterns, lack of sleep). Patients after liver transplantation constitute a specific group among patients with obesity. Apart from well–established risk factors for obesity development characteristic for the general population, liver recipients are at increased risk of numerous additional predisposing factors such as long–term immunosuppressive therapy, alteration in metabolism, all of which result in increased appetite and food intake, consequently fostering excessive accumulation of adipose tissue. Therefore, as expected, the incidence of both obesity and MS among liver transplant recipients significantly exceeds estimates for the general population.

The advancement in surgical techniques and optimisation of post-transplant care have resulted in a significant improvement in the short–term survival of liver recipients and transplanted organs. Unfortunately, analogous improvement was not observed in long–term observations. Suboptimal control over post–transplantation metabolic disorders are proposed as one of the key factors responsible for this phenomenon. Therefore, implementation of effective strategies for the prevention and treatment of metabolic complications could become a useful tool in optimising morbidity and mortality rates in this group of patients.

Aim

The aim of the study was to verify strategies in place for the monitoring of patients after liver transplantation in the context of metabolic complications and their impact on the short– and long–term survival of liver recipients, and to identify strategies to minimise metabolic risk.

3.1 Publication 1: Multidirectional facets of obesity management in the metabolic syndrome population after liver transplantation

Narrative Review

Metabolic syndrome is one of the most challenging global health concerns, prevalence of which reached epidemic levels in the general population secondary to the significant burden of obesity and DM2. Accordingly, MAFLD is rapidly increasing as an underlying cause of liver diseases and liver cirrhosis. Metabolic dysfunction–associated fatty liver disease in the post–transplantation setting may be a consequence of either the recurrence of the disease or its de novo development.

In this review, we discuss a broad range of clinical approaches that warrant attention to provide comprehensive and patient–centred medical care to liver transplant recipients, and to be prepared to confront the rapidly changing clinical challenges and ensuing dilemmas.

Healthy nutrition and physical activity are considered the cornerstone of obesity and MAFLD management in liver recipients. Effective weight reduction strategies are shown to alleviate liver steatosis, lead to MASH recovery, or benefit liver fibrosis. Unfortunately, many liver recipients are known to insufficiently respond to this approach. Few pharmacological options are available for treatment of obesity in the liver transplant population owing to the limited effectiveness and considerable adverse effects. Pharmacological armamentarium for MAFLD is even more limited. However, phase II of clinical trials with resmetirom has reported prospective results in MASH management. Bariatric surgery may be an alternative in eligible morbidly obese patients, who failed to respond to non–invasive therapeutic methods. Microbiota modifications with probiotics and prebiotics bring gratifying results in the management of metabolic complications and MASH. Faecal microbiota transplantation is successfully performed in many medical indications. However, the safety and efficacy profiles of faecal microbiota transplantation in immunocompromised patients remain unclear. Individualised immunosuppressive regimens are recommended following liver transplantation to address possible metabolic concerns. Regular revisions of prescribed and non–prescription medications should be in place to identify possible drug–drug interactions interfering with immunosuppression therapy, especially in patients with obesity as pharmacokinetic and/or pharmacodynamic properties of medicines may be altered in this group of individuals.

Effective and comprehensive management of metabolic complications is shown to yield multiple beneficial results in the liver transplantation population and may bring gratifying results in improving long–term survival rates.

3.2 Publication 2: Metabolic profile of liver recipients and determinants of their body fat distribution

Article

Obesity and DM2 epidemics exert a measurable impact on the liver transplantation population and translate into an evolving metabolic profile of patients with liver cirrhosis, increase the demand for liver transplantation and compromise long–term post–transplantation morbidity and mortality.

This study aimed to investigate the metabolic profile of liver recipients and its association with their body fat distribution.

We recruited 100 patients who underwent de novo elective cadaveric–donor liver transplantation. Metabolic complications were rare in the pre–transplantation period and displayed exponential growth following the liver transplantation procedure. Most of the pre–transplantation metabolic derangements continued post–transplant. Metabolic syndrome was identified in only 4% of liver transplantation candidates, the prevalence of which increased to 54% after the transplantation. Patients with post–transplantation MS presented unfavorable metabolic profile before transplantation and had significantly higher overall fat mass as well as abdominal fat accumulation, in both visceral and subcutaneous compartments. Patients who developed post–transplantation MS were characterised by a significantly higher serum concentrations of acute–phase reactants (C-reactive protein: 2.41 mg/dL vs. 1.21 mg/dL, p=0.05; serum ferritin concentration: 149.85 mg/dL vs. 53.55 mg/dL, p<0.001; for patients with

and without MS respectively), presented worse control over blood pressure (systolic blood pressure: 130 mmHg vs. 120 mmHg, $p<0.001$; diastolic blood pressure: 80 mmHg vs. 75 mmHg, $p=0.0075$; for patients with and without MS respectively) and carbohydrates homeostasis (fasting glucose: 105 mg/dL vs. 87.5 mg/dL, $p<0.001$; HOMA-IR: 1.14 vs. 0.86, $p<0.001$; HbA1c: 5.85% vs. 5%, $p<0.001$; for patients with and without MS respectively) as well as adverse lipid profiles (HDL: 50 mg/dL vs. 65 mg/dL, $p=0.0015$; triglycerides: 138.5 mg/dL vs. 97.5 mg/dL, $p<0.001$; for patients with and without MS respectively). The activity of liver function tests was significantly higher in the MS group when compared to the non–MS group (AST: 25.5 U/L vs. 19 U/L, $p=0.0033$; ALT: 25.5 U/L vs. 18U/L, $p=0.0012$; for patients with and without MS respectively). Patients with MS presented higher level of uric acid (6.55 mg/dL vs. 5.95 mg/dL, $p=0.0368$; for patients with and without MS respectively) and lower level of vitamin D3 (20.7 U/I vs. 34.12 U/I, $p<0.001$; for patients with and without MS respectively). None of the immunosuppressive regimens carried greater risk of MS development. A mean 6–month serum tacrolimus concentration showed higher values in the MS group compared to the non–MS group, but the result was statistically insignificant (6.18 ng/mL ± 1.44 vs. 5.67 ng/mL ± 1.5, $p=0.093$; for patients with and without MS respectively). An analysis of the tacrolimus serum concentration in subgroups (MS group and non–MS group) in relation to the applied maintenance immunosuppressive regimen did not show statistically meaningful differences either ($p=0.587$ and $p=0.367$, respectively). Neither prolonged steroid use ($p=0.14$) nor intravenous steroid administration in the management of acute organ rejection episodes ($p=0.282$) were associated with an increased risk of de novo MS. Glycated haemoglobin (OR: 8.962, 95% CI: 2.188–84.545, $p = 0.013$), ferritin (OR: 1.024, 95% CI: 1.005–1.054, $p = 0.038$), and post–transplantation hypertriglycaeridemia (OR 27.957, 95% CI: 2.626–752.121, $p = 0.014$) were found to be independently associated with de novo MS. Of the body composition parameters, only amount of VAT and SAT correlated with the increased risk of MS development ($p=0.021$ and $p=0.045$, for amount of VAT and SAT, respectively). Yet, the association was not confirmed in the multivariate analysis. Of liver transplant indications, only hepatitis C infection promoted both visceral and subcutaneous adipose tissue accumulation ($p=0.0021$ and $p=0.0023$; for amount of VAT and SAT, respectively). Almost all of post–transplantation metabolic complications significantly correlated with the greater accumulation of abdominal adipose tissue. None of the immunosuppressive schemes ($p=0.3625$, $p=0.6638$; for amount of VAT and SAT, respectively) nor chronic steroid use ($p=0.0843$, $p=0.2393$; for amount of VAT and SAT, respectively) influenced abdominal fat distribution. Of the biochemical markers, serum level of uric acid and vitamin D3 showed an association with VAT; however, the strength of the correlation was low. A low to moderate association was noted with serum ferritin concentration ($p<0.001$, $p<0.001$, for amount of VAT and SAT, respectively) and the parameters of carbohydrates metabolism and both abdominal fat compartments (HbA1c: $p<0.001$, $p<0.001$; insulin: $p=0.001$, $p=0.014$; HOMA-IR: $p<0.001$, $p=0.010$; for amount of VAT and SAT, respectively). Only the anthropometric obesity indices were significantly associated with abdominal fat distribution in liver recipients in multivariate analysis.

Despite the relatively young age of liver donor (mean age 38 years), 34% of them were found with overweight and 6% with obesity based on BMI calculation. Abdominal obesity was present in 34% of liver donors. Whereas excessive accumulation of overall as well as abdominal fat in liver donors was found to be associated with an increased risk of MS and obesity development in liver recipients.

Importantly, independent risk factors identified for post–transplantation MS and the pattern of abdominal fat distribution did not correspond, which underpins the complex and multicausal

nature of both metabolic complications and abdominal fat distribution resulting from the multifaceted interplay between genetic, environmental, behavioural, social and iatrogenic factors. In contrast to previous reports, 74.2% of study participants were diagnosed with obesity. This may be partially explained by the timing of the study, which was conducted during the COVID-19 pandemic. During this difficult time, imposed restrictions and social isolation resulted in reduced physical activity, adverse nutritional habits, and comfort eating.

In conclusion, metabolic complications were common in liver recipients. In order to abrogate the metabolic risk in the post–transplantation setting, a personalised risk assessment and the monitoring of liver recipients are strongly recommended. Appropriate precautionary measures should be applied to prevent weight gain should another unprecedented health emergency arise.

3.3 Publication 3: MASH continues as a significant burden on metabolic health of liver recipients

Article

Metabolic complications are a recognized health concern in liver transplantation recipients that result in inferior patients–reported outcomes. Patients with MASH are known to be disproportionately affected by metabolic diseases compared to patients with other indications for transplantation.

The aim of this study was to investigate the incidence and time of onset of metabolic abnormalities and weight gain trajectory of liver recipients with specific focus on differences between patients transplanted for MASH and non–MASH causes.

An observational, monocentric and retrospective analysis was performed. Patients who received a cadaveric–donor–liver transplantation between 2010 and 2019 were eligible.

Post–transplantation MS was diagnosed in 36% (n=96) of the study population, with over half of the cases identified during the first year after transplantation. During a median follow–up of 89.6 months, 62.4% (n=151) patients developed post–transplantation hypertension, 48.5% (n=98) developed post–transplantation DM2 and 47% (n=77) developed de novo dyslipidaemia. During the observation period, a sharp increase in metabolic complications was observed within the first year of follow–up (45% of dyslipidaemia cases, 77.5% of DM2 cases, 76.8% of hypertension cases), with subsequent stabilization over the 3rd year of observation. Patients that underwent transplantation owing to MASH (8.2%, n=23) showed a significantly higher incidence of metabolic complications in both pre– and post–transplantation period. We documented a rapid weight increase, which was most pronounced in the early post–transplantation period and in patients with underlying MASH, rendering many recipients overweight or obese. Body mass index values continued to grow until the 3rd year of observation until they reached plateau. Although BMI trend patterns have been predominantly shared by patients with and without underlying MASH, individuals that underwent transplantation for MASH showed higher BMI values at baseline and were more frequently overweight and obese as liver transplantation candidates. In the long–term observation, BMI values in patients with MASH aetiology of liver disease showed non–statistically significant decrease between the 5th and 10th year of observation, but still remained higher compared to patients with non–MASH–related liver diseases in the corresponding period of time. Individuals transplanted for non–MASH causes

failed to show beneficial weight reduction in long–term observations. Instead, their weight stabilised after the 3rd year of observation and remained intact thereafter. Notably, MASH was also found to be an independent predictor of post–transplantation MS increasing its risk by 5.5 times (p=0.01). Once MASH was accompanied by post–transplantation weight gain, the risk increased further by 32.1% per each BMI point (p<0.001).

Despite the magnitude of the problem and seemingly well–defined metabolic areas of concern, managing MASH still represents a significant challenge for healthcare professionals and patients. Liver recipients with underlying MASH significantly surpassed patients transplanted for other indications in terms of metabolic complications incidence and demonstrated an unfavourable trajectory of weight gain post–transplantation. Keeping in mind that metabolic status translates into numerous aspects of human health and further determines post–transplantation prognosis, patients transplanted due to MASH warrant heightened attention during pre–transplantation work–up which should be continued during post–transplantation observation.

3.4 Publication 4: De novo metabolic syndrome 1 year after liver transplantation and its association with mid- and long-term morbidity and mortality in liver recipients

Article

Metabolic syndrome constitutes an important source of CV and cancer–related morbidity and mortality in the general population. Limited information is available on whether these findings can be directly extrapolated to liver recipients.

This study aimed to investigate the impact of post–transplantation MS present one year after liver transplantation on mid– and long–term survival rates, risk of major CVEs, and de novo malignancies in deceased–donor–liver recipients.

Adult deceased–liver–donor recipients who underwent transplantation in our centre between 2010 and 2019 and reached at least one year of post–transplantation follow–up were eligible. The risk of death, major CVEs and de novo malignancies was evaluated using multivariate Cox regression models adjusted for their respective traditional risk factors. Proportional hazard models adjusted for sex, age at liver transplantation, MASH as aetiology of liver disease, tobacco use, and alcohol abuse were constructed to evaluate the risk of major CVEs and de novo malignancies in patients with and without new-onset MS at 1 year post-transplantation. The risk of death was evaluated using multivariate Cox regression models adjusted for sex, age at liver transplantation, MELD score and eGFR as possible confounders.

Of 259 enrolled patients, 20% (n=52) developed post–transplantation MS one year after the procedure. At one year, a sizable proportion of liver recipients were still maintained on low–dose steroids, with only 32.5% of patients successfully converted to steroid–free regimens with tacrolimus monotherapy or CNI in combination with MMF. Based on adjusted Cox regression analysis, MS did not increase the overall risk of death in liver recipients (HR: 1.165; 95% CI: 0.842–3.24, p=0.144). Nevertheless, Kaplan–Meier survival curves derived from the Cox regression models demonstrated a trend for inferior overall survival among patients who developed MS, with survival rates of 94.5%, 88.4%, and 70.2%, and 96.7%, 92.8%, and 80.8% for patients with and without post–transplantation MS at 3, 5, and 10 years, respectively (p=0.029). Development of MS was associated with an overall (HR: 2.82; 95% CI: 1.174–6.76, p=0.02) and time-dependent increase in the risk of major CVEs (p<0.001). Cumulative risks of de novo tumours at 3, 5, and 10 years did not show significant differences between patients who

developed MS at one year after liver transplantation and those without the condition (p=0.198). Metabolic dysfunction–associated steatohepatitis aetiology of liver disease (HR: 4.7; 95% CI: 1.386–16.071, p=0.012), pre–existing major CVE (HR: 18.514; 95% CI: 3.196–156.375, p=0.002), and development of de novo malignancy (HR: 3.908; 95% CI: 1.524–9.956, p=0.004) were independent predictors of all–cause mortality in liver recipients. Noteworthy, despite the routine surveillance strategy in place, de novo tumours constituted the second most frequently reported cause of death in our population. Age at liver transplantation (HR: 1.135; 95% CI: 1.061–1.1229, p<0.001), tobacco use (HR: 9.169; 95% CI: 1.948–48.270, p=0.006), and post–transplantation MS at one year (HR: 4.091; 95% CI: 1.141–15.694, p=0.033) were associated with increased risk of major CVEs after transplantation. Maintenance of GSK at one year post–transplantation and CSA use increased the risk of de novo tumours by approximately 6.91 (p=0.036) and 5.35 times (p=0.009), respectively. The overall duration of GSK exposure did not affect the risk of post–transplantation carcinogenesis (p=0.799). Risk factors commonly associated with increased risk of cancer development, such as alcohol abuse (p=0.678),tobacco use (p=0.948), and history of malignancy (p=0.988), were not associated with increased risk of de novo tumours in liver recipients.

The study demonstrated that evidence originating from the general population cannot be directly extrapolated to the special population of liver recipients. Obtained results indicate that transplant–specific factors significantly modulate the effect of MS on post–transplantation outcomes and outweigh the impact of traditional risk factors in terms of carcinogenesis. Our results weigh in favour of tacrolimus–based immunosuppression to mitigate cardiac– and cancer–related morbidity compared to CSA–based regimens. Considering the wide–ranging effects of MS on post–transplantation prognosis, it is of paramount importance to put emphasis on the prevention, early recognition, and adequate management of MS and all its modifiable constituents in order to improve the late outcomes of liver recipients.

3.5 Publication 5: Body mass index: an unreliable adiposity indicator for predicting oncological outcomes in liver recipients with HCC in a native liver

Article

Obesity is a well–documented and modifiable risk factor for the development of HCC in the general population. The applicability of these findings to liver recipients is uncertain, and results of available data have not been unanimous.

The objectives of the current study were to investigate the association between the pre–operative dry–weight–adjusted BMI, as a surrogate measure of obesity, and oncological outcomes in liver recipients with HCC in a native liver.

This observational, retrospective study enrolled all patients with histologically confirmed HCC in a native liver who underwent liver transplantation from a deceased donor in our center between 2008 and 2018. Patients were stratified according to their pre–operative BMI into three groups: patients with normal body weight (n=53), patients with overweight (n=23), patients with obesity (n=7). Oncological outcomes were defined as risk of 5–years OS, 5–year RFS, and risk of HCC recurrence.

Overall, 19.3% (n=16) of the patients died during a median follow–up of 60 months. Tumour recurrence occurred in 12% (n=10) of study participants. Dry–weight–adjusted BMI failed to predict the 5–year RFS (p=0.55), risk of tumour recurrence (p=0.314) and 5–year OS (p=0.19) in liver recipients. Neither BMI at one year follow–up (p=0.314; p=0.2667, for the risk of HCC

recurrence and OS, respectively) nor a weight increase between baseline and one year post–transplantation (p=0.721; p=0.3621, for the risk of HCC recurrence and OS, respectively), when the weight increase tended to be the most pronounced, showed an association with an increased risk of HCC recurrence or OS. Pre–existing DM2 did not impact the risk of either OS (p=0.1791) or HCC recurrence (p=0.462). Tumour recurrence constituted the sole determinant of 5–year OS (HR: 13.961; 95 CI 3.442-56.6; p<0.001), whereas risk of HCC recurrence was independently associated with fulfilment of the Milan criteria, which decreased the risk of relapse by approximately seven times (p=0.46). Presence of histologically confirmed microvascular invasion was associated with an approximately 25–fold increase in the risk of HCC recurrence (p=0.01), whereas increase in AFP level by one point increased this risk by approximately 1.41 times (p=0.03).

Body mass index was proven to be an unreliable surrogate measure of obesity for predicting oncological outcomes among liver transplant recipients. Other obesity indices should be referenced in order to assess cancer–related prognosis more accurately in liver recipients.